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Synthesis of cyclic RGD derivatives via solid phase macrocyclization using the Heck reaction

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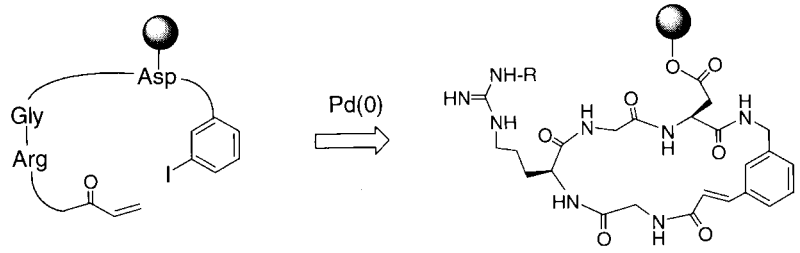
Abstract—A novel intramolecular macrocyclization reaction on a solid support using the Heck reaction has been achieved. For head to tail cyclization on a solid support, the linear precursor was anchored to a chlorotrityl chloride resin via an ester linkage using the β-carboxyl group of Asp. The Heck coupling of acrylic acid amide to 3-iodobenzylamine on the solid support proceeds smoothly to yield a cyclic tetrapeptide derivative, which contains a new 3-substituted cinnamic acid template and Arg-Gly-Asp sequence. The macrocyclization reaction takes place considerably more rapidly on a solid support than in solution. The solid phase procedure was successfully used for the construction of cyclic RGD libraries having diverse side chain structures, combined with a variety of ring sizes.

The application of solid support chemical synthesis has recently been extended beyond the traditional preparation of biopolymers to the synthesis of general organic molecules, for the preparation of chemical libraries in combinatorial synthesis.[1] In order for solid phase synthesis to be useful in generating diversity, however, the repertoire of carbon–carbon bond forming reactions that can be used in the preparation of biologically relevant molecules need to be increased. The Heck reaction[2] provides a very attractive method for the preparation of such bonds on solid support. The reaction does not require a stoichiometric amount of Pd(0) nor inert anhydrous conditions and proceeds readily at room temperature, even on solid support.[3] The intramolecular Heck reaction is a useful method for the preparation of five, six, or seven-membered rings fused to aromatic rings. Although the reaction has been applied to the solid phase synthesis of indole derivatives,[4] benzofuran derivatives,[5] and isoquinolinone derivatives,[6] the application of these synthetic strategies to the production of more complex macrocyclic molecules have, to date, been rather limited in scope.[7]

We selected intramolecular macrocyclization as a suitable reaction that could be carried out efficiently on a solid support rather than in solution, because of the 'pseudo-dilution' effect.[8] This specific effect, which is the result of the polymeric solid support, has been used successfully for macrocyclization via disulfide bond formation in peptide chemistry.[9] In contrast to these solid phase results, infinite dilution is generally inevitable in solution phase reactions,



Scheme 1.

Keywords: RGD derivatives; Heck reaction; cyclic tetrapeptide; solid phase macrocyclization; *tert*-alcohol resin.
Abbreviations: Bu^t, *tert*-butyl; CIP, 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate; DIPCDI, diisopropylcarbodiimide; HRFAB, high-resolution fast atom bombardment; Fmoc, fluoren-9-ylmethyloxycarbonyl; HOAt, 1-hydroxy-7-azabenzotrizazole; HOBt, 1-hydroxybenzotriazole; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; MD, molecular dynamics; MS, mass spectrometry; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; SPPS, solid phase peptide synthesis; TFA, trifluoroacetic acid; TFE, trifluoroethanol.
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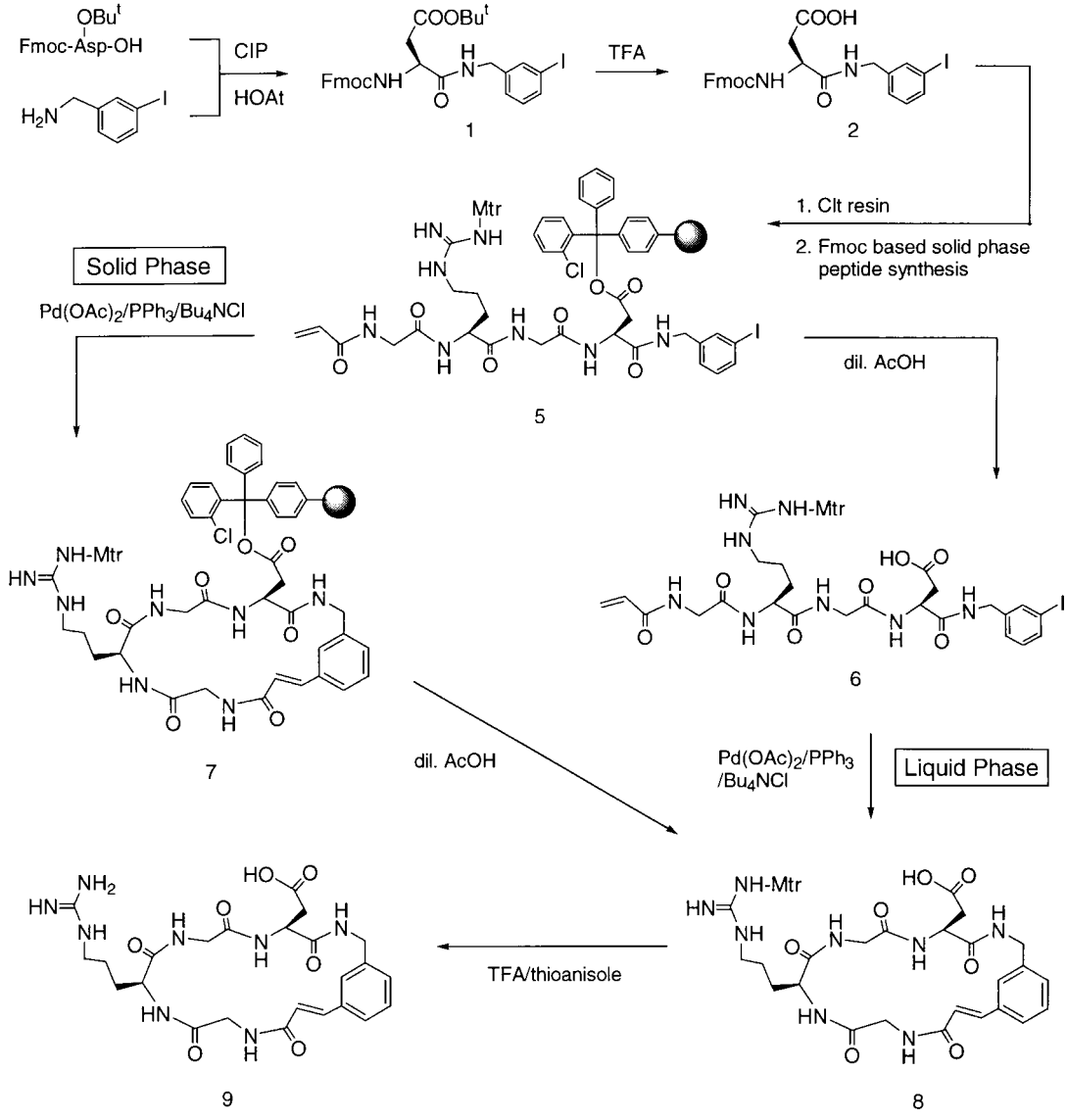
for obtaining a reasonable reaction efficiency of the monomeric product. Based on the above information, we report here a novel intramolecular macrocyclization on a solid support using the Heck reaction.[10]

A cyclic tetrapeptide derivative was synthesized using the Heck coupling of acrylic acid amide to a 3-iodobenzyl amine moiety, on a solid support. The cyclic derivative consists of a new 3-substituted cinnamic acid template for the construction of the rigid cyclic structure containing Arg-Gly-Asp (RGD) sequence (Scheme 1). The RGD sequence is a common recognition motif for the integrin family of receptors, which are involved in cell–cell and cell–matrix adhesion.[11] Glycoprotein IIb/IIIa (GP IIb/IIIa), a member of the integrin family, is expressed on the surface of activated platelets which binds to fibrinogen to cause platelet aggregation. Thus, the solid phase preparation of cyclic RGD-derivatives with affinities for GPIIb/IIIa could be effective approach for the discovery of drug candidates which could prevent the formation of platelet-rich clots.[12]

1. Results and discussion

The synthetic scheme for the cyclic RGD derivative is outlined in Scheme 2. For the construction of the linear precursor peptide, a base-labile Fmoc group was employed as the N^{α}-protecting group.[13] The linear precursor is anchored to a solid support through the β-carboxyl group of Asp; this makes intramolecular head to tail cyclization on the solid support feasible. After the cyclization, the desired product can be cleaved from the solid support by treatment with mild acid. Based on this scheme, we first examined a suitable solid support for precursor synthesis and reaction efficiency of solid phase intramolecular cyclization.

For anchoring, a *C*-terminal Asp derivative, **2**, was prepared via the CIP-mediated coupling[14] of commercially available Fmoc-Asp(OBu^t)-OH and 3-iodobenzylamine followed by cleavage of the side chain Bu^t group with TFA. The use of the conventional Wang resin[15] and more acid-labile 2-chlorotrityl resin (Clt resin)[16] were examined as potential solid supports. To the Wang resin, at first, the Asp derivative



Scheme 2.

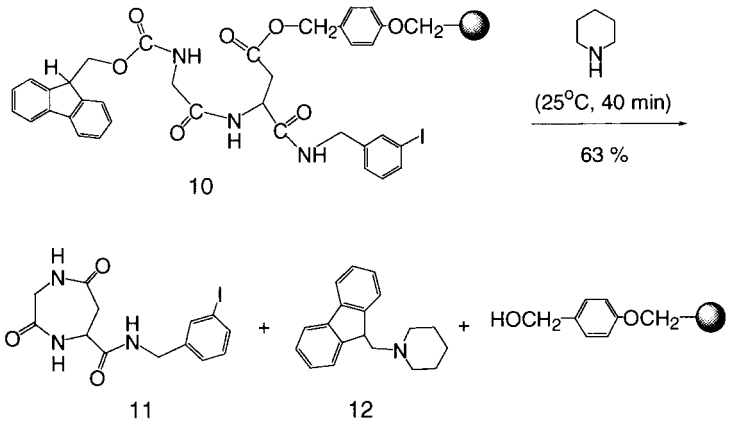


Figure 1. Dipeptide cleavage by intramolecular aminolysis of the β-benzyl ester linked to the Wang resin. Cleavage yield was estimated by amino acid analysis of the recovered resin.

prepared above was anchored according to literature procedures. The successive introduction of Fmoc-Gly-OH and Fmoc-Arg(Mtr)-OH[17] was then conducted by the combination of piperidine mediated Fmoc deprotection and DIPCDI mediated coupling. However, no increment of the resin weight was obtained because of the dipeptide cleavage by intramolecular aminolysis of β-benzyl ester linked to the solid support (Fig. 1). After treating with piperidine, the diketodiazepin **11** and *N*-(fluoren-9-ylmethyl)piperidine **12** were isolated and characterized from the filtrate. The cleavage yield was estimated at 63% by amino acid analysis of the recovered Wang resin.

We then examined the construction of the desired precursor starting from the Clt resin. The Asp derivative **2** was anchored to the Clt resin according to the published procedure. The desired precursor was then constructed by the successive introduction of Fmoc-Gly-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Gly-OH, and acrylic acid monomer by the combination of piperidine mediated Fmoc deprotection and DIPCDI mediated coupling. Each condensation reaction was monitored by the Kaiser-ninhydrin test.[18] The quantitative increment of the resin weight was obtained and the incorporation of amino acids was confirmed by amino acid analysis of the product resin. In addition, the linear precursor **6** obtained by a brief treatment of the precursor resin with a mixture of AcOH–TFE–CH_2Cl_2 (1:1:2) showed a sharp main peak on analytical HPLC. Thus, in the present synthesis, the linear precursor for head to tail cyclization was successfully synthesized from the sterically hindered Clt resin.

The efficiency of solid phase macrocyclization was then investigated and the results were compared with that in solution phase. The linear precursor resin **5** obtained above was treated with $Pd(OAc)_2$ in the presence of Ph_3P and Bu_4NCl using DMF/H_2O/Et_3N as the solvent. The cyclization reactions were conducted for 2, 4, and 8 h in parallel at 37°C. Each resin was then treated with AcOH–TFE–CH_2Cl_2 (1:1:8) and the detached product was analyzed using HPLC to examine the progress of the reaction. For comparison, a cyclization reaction in solution was also conducted. The linear precursor **6**, obtained by AcOH–TFE–CH_2Cl_2 treatment of the precursor resin **5**, was treated with $Pd(OAc)_2$/Ph_3P/Bu_4NCl in DMF/H_2O/Et_3N under the same reaction conditions as described in the solid phase reaction. An aliquot was periodically removed from the reaction mixture and the progress of the reaction was monitored in a similar manner by HPLC.

The intramolecular cyclization in solution proceeded in proportion to the reaction time, but was relatively slow. Most of precursor **6** was converted to the cyclized product **8** after 8 h of reaction (Fig. 2b). In contrast, the same cyclization reaction occurred rapidly on the solid support as shown in Fig. 2a. Most of the precursor was converted to product within 2 h and the time course of the reaction was quite different with that of solution phase reaction. The results clearly show that the Pd(0)-mediated intramolecular macrocyclization described in this paper is one of the reactions suitable for solid phase organic synthesis.

Thus, for the preparation of a cyclic RGD derivative **9**, the Pd(0)-mediated macrocyclization of **5** employing $Pd(OAc)_2$ with Ph_3P and Bu_4NCl in a DMF/H_2O/Et_3N solvent system was carried out at 37°C for 4 h to provide **7**. The cyclized peptide resin **7** was then treated with AcOH–TFE–CH_2Cl_2 to cleave the protected cyclic tetrapeptide **8** from the resin support. The cyclic product **8** was treated with TFA–thioanisole at 25°C for 3 h without any further purification to remove the Mtr group from the Arg side chain. The deprotected product **9** was purified by preparative HPLC, and the homogeneous product was obtained in 20% overall yield (calculated from the starting resin). The purified cyclic derivative **9** exhibited a single sharp peak on analytical HPLC and was shown to be a monomer by HRFAB MS. The *trans*-configuration of the product **9** was defined by ^1H NMR analysis.

The purified cyclic RGD derivative inhibited fibrinogen binding to immobilized GPIIb/IIIa with IC50 2.0×10^{-5} M, but showed no inhibitory activity for the vitronectin receptor, another member of the integrin family of receptors. According to the works on active conformation of RGD peptides,[19] the distance between the guanidino group of Arg and the β-carboxylic acid of Asp should be within 12–18 Å to show platelet aggregation activity. From a ROESY (rotating-frame Overhauser enhancement spectroscopy) experiment of the purified cyclic product and molecular dynamics (MD) simulations, the distance between the

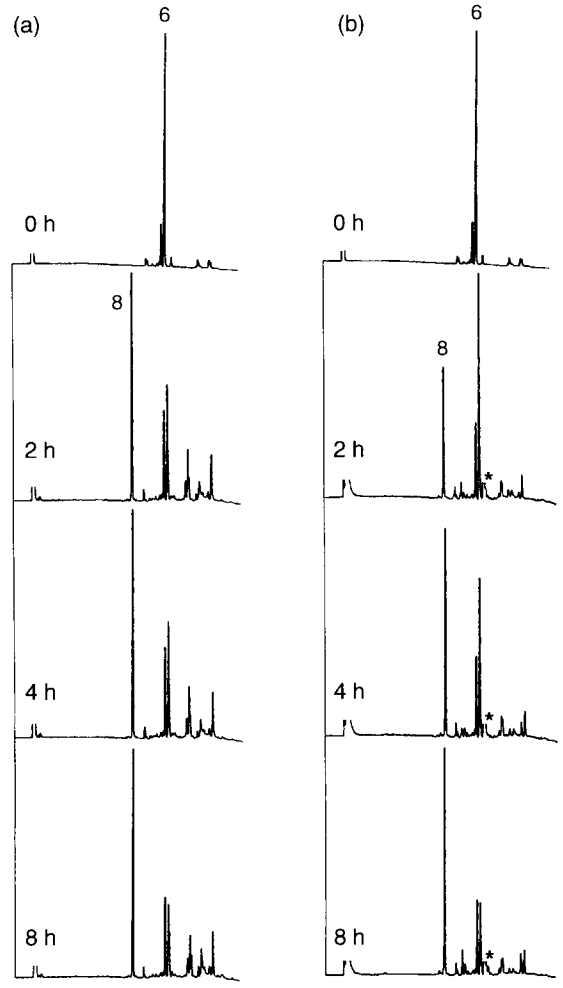


Figure 2. HPLC profiles of the cyclized products on solid support: (a) or in solution, (b) *derived from Ph_3P. HPLC on YMC AM302 [CH_3CN (20–30%/30 min) in 0.1% aq. TFA, 0.9 ml/min]

guanidino group and β-carboxyl group in **9** was determined to be within 12–15 Å in its typical lower energy conformation. Thus, presumably, conformational restriction of the cyclized product **9** contributed, to some extent, to its selectivity for integrin receptors.

The above solid phase macrocyclization reaction was then applied to library construction by the combinatorial approach because the 3-substituted cinnamic acid template containing the RGD sequence showed a weak but specific binding to GPIIb/IIIa. Two sites were selected for introducing structural diversity: one at the side chain of the *N*-terminal amino acid residues to alter the steric or ionic effect and the second at the residue between Arg and Asp, to alter the ring size. To facilitate the synthesis, the split and mix procedure[20] was selected for the preparations of these libraries.

Based on the above scheme, we first constructed an alkyl library, which consisted of 15 components (Fig. 3). To introduce diversity regarding steric effect in this library, proton, methyl, ethyl, propyl, and cyclic propyl structures were incorporated at the side chain of the *N*-terminal amino acid. For this purpose, each of the corresponding amino acids, Gly, Ala, α-aminobutylic acid (αAbu), norvaline (Nva), or Pro was introduced. The ring size was altered by inserting different numbers of methylene groups between the Arg and Asp residues, i.e. by introducing Gly, γ-aminobutylic acid (γAbu), or ϵ-aminohexanoic acid (ϵAhx) residues at this position.

For multi component macrocyclization, the alkyl library precursor resin was treated with $Pd(OAc)_2/Ph_3P/Bu_4NCl$ under the same reaction conditions as described for the monomeric resin. The solid phase reaction was easily monitored on HPLC by analyzing the acid extract of the starting and product resins. Each component prior to and after cyclization was unambiguously identified by MALDI–TOF MS

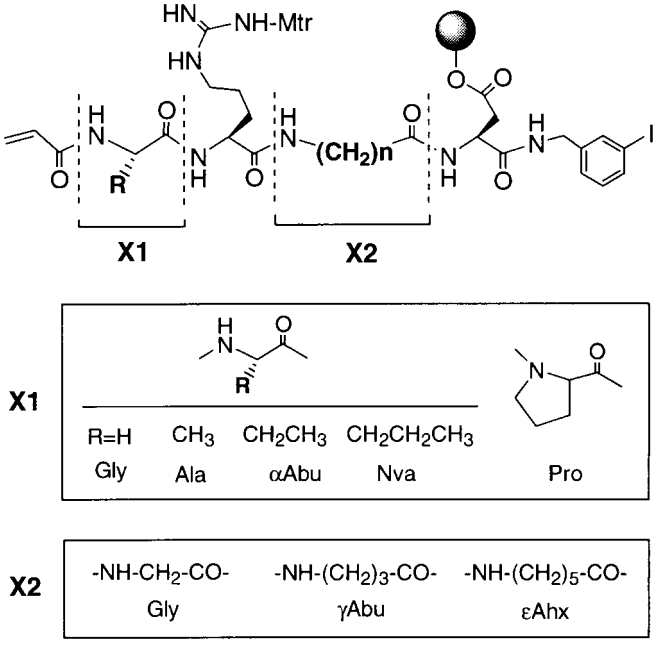


Figure 3. Alkyl library.

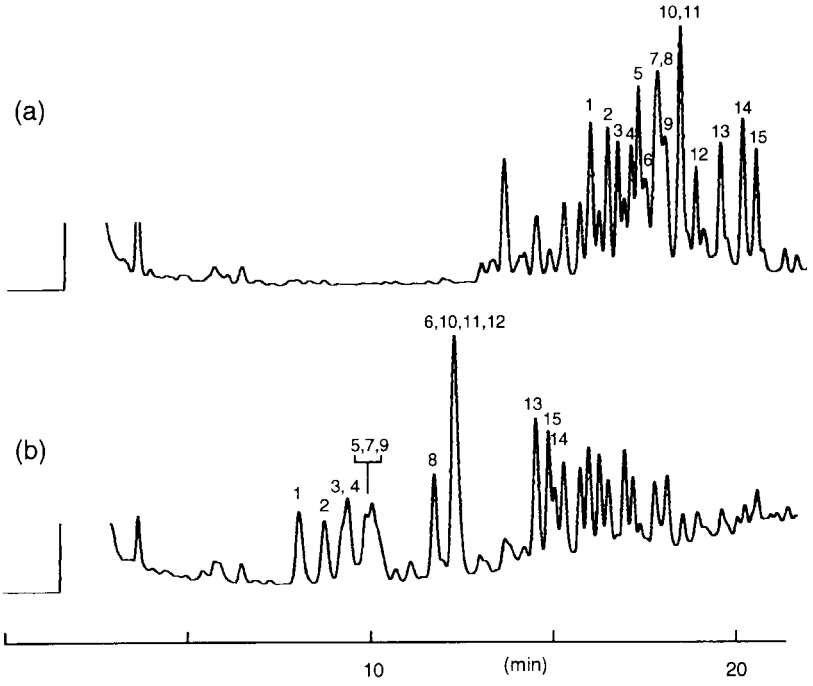


Figure 4. HPLC profiles of the alkyl library prior to (a) and after (b) cyclization. Each component was identified by MS-analysis: 1 [Gly[1], γAbu[3]], 2 [Gly[1], Gly[3]], 3 [Ala[1], γAbu[3]], 4 [Gly[1], ϵAhx[3]], 5 [Ala[1], Gly[3]], 6 [Pro[1], γAbu[3]], 7 [αAbu[1], γAbu[3]], 8 [Ala[1], ϵAhx[3]], 9 [Pro[1], Gly[3]], 10 [αAbu[1], Gly[3]], 11 [Pro[1], ϵAhx[3]], 12 [αAbu[1], ϵAhx[3]], 13 [Nva[1], γAbu[3]], 14 [Nva[1], Gly[3]], and 15 [Nva[1], ϵAhx[3]] derivative.

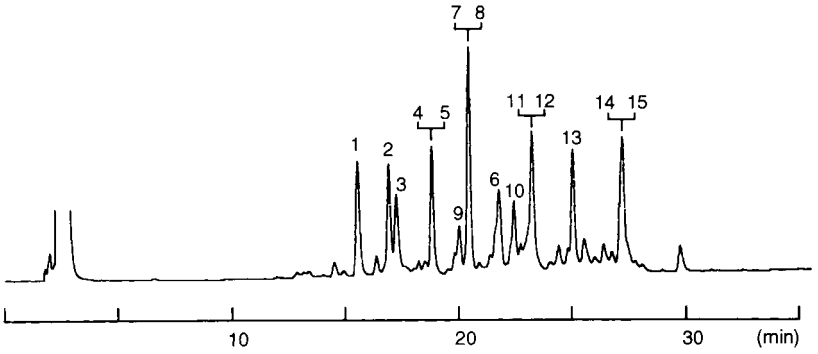
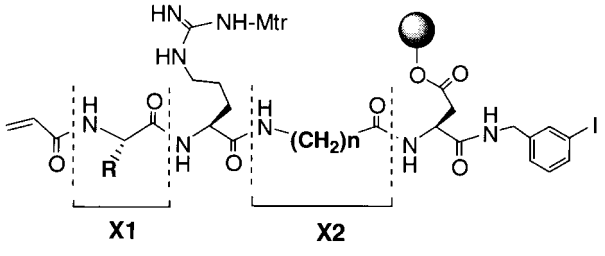


Figure 5. HPLC profile of the alkyl library after deprotection. For identification of each compound, see the caption of Fig. 4.

analysis of each peak, although both chromatograms showed relatively complex elution patterns (Fig. 4). No other peptide components were detected from the chromatogram. The cyclized product was cleaved from the resin with AcOH/TFE/DCM and the Mtr group of the product was removed by TFA/thioanisole as described for cyclic RGD derivative **9**. The deprotected mixture was quickly passed through a reversed phase HPLC column to remove hydrophobic contaminants such as scavengers, which are eluted at high concentrations of CH_3CN. The desired library product, which consisted of 15 components, was obtained as white powder in 10% overall yield calculated from the starting resin. Each component of the library was easily identified by HPLC (Fig. 5) and MS analysis.

The same approach was then applied to the construction of a functional group library (Fig. 6). Instead of alkyl group in the above described alkyl library, four types of functional groups, hydroxyl, amino, carboxyl, and amido groups, were incorporated to introduce diversity regarding ionic



X1	R				
	CH_2OH	CH_2NH_2	$(CH_2)_4NH_2$	CH_2COOH	CH_2CONH_2
	Ser	Dap	Lys	Asp	Asn

X2	-NH-CH_2-CO-	-NH-$(CH_2)_3$-CO-	-NH-$(CH_2)_5$-CO-
	Gly	γAbu	ϵAhx

Figure 6. Functional group library.

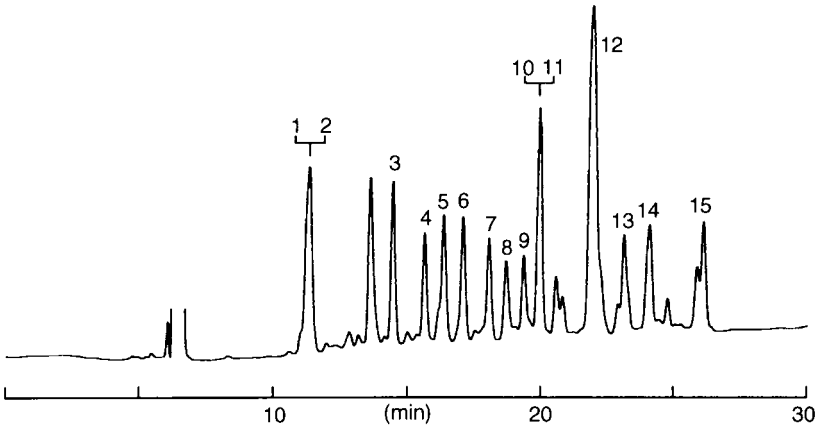


Figure 7. HPLC profile of the functional group library. Each component was identified by HRFAB MS analysis: 1 [Dap[1], γAbu[3]], 2 [Dap[1], Gly[3]], 3 [Lys[1], γAbu[3]], 4 [Lys[1], Gly[3]], 5 [Dap[1], ϵAhx[3]], 6 [Asn[1], γAbu[3]], 7 [Ser[1], γAbu[3]], 8 [Asn[1], Gly[3]], 9 [Ser[1], Gly[3]], 10 [Asp[1], γAbu[3]], 11 [Lys[1], ϵAhx[3]], 12 [Asp[1], Gly[3]], 13 [Asn[1], ϵAhx[3]], 14 [Ser[1], ϵAhx[3]], and 15 [Asp[1], ϵAhx[3]] derivative.

effects. For this, each of the corresponding amino acids, Ser, diaminopropionic acid (Dpr), Lys, Asp, or Asn were introduced. Combined with ring size diversity, this library also consists of 15 components. The construction of the precursor resin by the split and mix procedure and the following solid phase macrocyclization were conducted under the same conditions as described for the alkyl library. All components prior to and after cyclization were similarly identified by HPLC-MS analysis without difficulties. After partial purification by HPLC, the desired product, which consisted of 15 components was obtained as white powder in 9% overall yield. Each component in the library was similarly identified by HPLC-MS analysis without difficulty (Fig. 7).

2. Conclusion

The finding here clearly demonstrate that the Heck reaction can be used for solid phase macrocyclization reaction. The *tert*-alcohol based Clt resin was more suitable than the benzyl alcohol based Wang resin for the preparation of the linear precursor used for head to tail cyclization. In addition, the acid lability of the Clt resin was especially advantageous for monitoring the solid phase reaction by HPLC-MS analysis. The monitoring method showed that the reaction proceeded more efficiently on the solid support than in solution, presumably because of the pseudo-dilution effect. Thus, the cyclic RGD derivative and its libraries were easily prepared, isolated, and identified by applying the solid phase macrocyclization combined with HPLC-MS analysis. Preparation of a variety of libraries followed by an assessment of fibrinogen inhibitory activity will allow the application of this solid phase procedure to the design of a high affinity ligand of GPIIb/IIIa.

3. Experimental section

3.1. General

Solvents were reagent grade and dried prior to use. Fmoc amino acid derivatives, except for the diaminopropionic acid (Dpr) derivative, the Wang resin, and the 2-chlorotrityl chloride resin were obtained from Calbiochem Novabiochem and were used without further purification. Fmoc-Dpr(Boc)-OH was prepared according to the procedure described by Waki et al.[21] For quantification of Fmoc amino acids on the resin, the absorbance at 301 nm after cleavage of the Fmoc group with piperidine was measured according to the procedure described by Meienhofer et al.[22]

Melting points were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a 270 MHz (JEOL), 400 MHz (Bruker), or 600 MHz (Bruker) spectrometer with TMS as an internal standard. HRFAB MS was obtained on a JEOL JMS-SX102A spectrometer equipped with a JMA-DA7000 data system. MALDI-TOF MS was obtained on a Voyager-DE Biospectrometry Workstation of PerSeptive Biosystems. The binding of fibrinogen to immobilized human GP IIb/IIIa was performed according to the method of Hayashi et al.[23] Molecular modeling studies were performed using Insight and Discover programs. Molecular dynamics (MD) simulations and energy minimization (EM) were conducted using consistence valence force field (CVFF) parameters according to the method of Siahaan et al.[24] HPLC was carried out on a reversed phase column which was eluted with CH_3CN in 0.1% aqueous TFA and detected at OD 220 nm: Rt_1–Rt_4, YMC AM302 (4.6×150 mm), flow rate 0.9 ml/min; Rt_5, YMC-Pack ProC18 AS 323 (10×250 mm), flow rate 2.5 ml/min.

3.1.1. Fmoc-Asp(OBu^t)–NH–CH$_2$–C$_6$H$_4$–I, 1. To a solution of Fmoc-Asp(OBu^t)–OH (0.50 g, 1.2 mmol) in THF (5.0 ml) were added DIEA (0.63 ml, 3.6 mmol), HOAt (0.10 g, 0.74 mmol), CIP (0.41 g, 1.5 mmol), and 2-iodobenzylamine (0.19 g, 1.4 mmol), and the mixture was stirred at 25°C for 45 min. AcOEt (20 ml) was then added to the mixture and the organic phase was washed with 5% citric acid, H_2O, dried over $MgSO_4$, and evaporated. The crude product was purified by silica gel column chromatography using $CHCl_3$ followed by precipitation with AcOEt/hexane to yield 0.66 g (87%) of **1** as a solid: Mp 89–90°C,$[\alpha]_D^{25}=-14°$ (*c* 0.50, DMF), ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.76–7.73 (m, 2H), 7.60–7.55 (m, 4H), 7.43–7.26 (m, 4H), 7.20 (d, *J*=7.6 Hz, 1H), 7.02 (t, *J*=7.6 Hz, 1H), 6.83 (br t, *J*=6.3 Hz, 1H), 5.96 (d,

J=8.0 Hz, 1H), 4.53 (m, 1H), 4.45 (br d, J=6.3 Hz, 2H), 4.38 (m, 2H), 4.20 (t, J=6.6 Hz, 1H), 2.96 (dd, J=17.2 Hz, J=3.8 Hz, 1H), 2.61 (dd, J=17.2 Hz, J=6.6 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.28, 170.49, 156.13, 143.61, 141.35, 140.34, 136.57, 136.40, 130.35, 127.80, 127.10, 126.74, 124.99, 120.07, 94.55, 82.07, 67.17, 51.18, 47.17, 42,75, 37.34, 28.07. Anal. Calcd for $C_{30}H_{31}N_2O_5I$: C, 57.51; H, 4.99; N, 4.47. Found: C, 57.67; H, 5.12; N, 4.31. HRFAB MS, m/z 627.1359 for $[M+H]^+$ (Calcd 627.1356 for $C_{30}H_{32}N_2O_5I$).

3.1.2. Fmoc-Asp–NH–CH_2–C_6H_4–I, 2. TFA/anisole (22–2.2 ml) was added to Fmoc-Asp(OBu^t)–NH–CH_2–C_6H_4–I (2.5 g, 4.0 mmol) and the mixture was stirred at 25°C. During the stirring, a flocculent precipitate separated out. After 90 min of stirring, TFA of the mixture was removed by evaporation at 20°C. Hexane (50 ml) was added to the residue and the precipitate was filtered and dried to yield 2.0 g (88%) of **2** as a white powder. The product was used immediately for anchoring without purification. Mp 181–182°C, $[\alpha]_D^{25}=-12°$ (c 0.50, DMF), ^{1}H NMR (400 MHz, DMSO-d6) δ8.47 (t, J=5.9 Hz, 1H), 7.89 (d, J=7.5 Hz, 2H), 7.72 (d, J=7.7 Hz, 3H), 7.62 (s, 1H), 7.58 (d, J=7.8 Hz, 1H), 7.42 (t, J=7.4 Hz, 2H), 7.33 (t, J=7.3 Hz, 1H), 7.32 (t, J=7.4 Hz, 1H), 7.25 (d, J=7.7 Hz, 1H), 7.09 (t, J=7.7 Hz, 1H), 4.38 (m, 1H), 4.34–4.21 (m, 5H), 2.70 (dd, J=16.5, 5.3 Hz, 1H), 2.53 (dd, J=16.5, 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d6) δ171.72, 170.88, 155.84, 143.77, 142.12, 140.69, 135.56, 135.37, 130.37, 127.64, 127.09, 126.42, 125.34, 120.10, 94.70, 65.81, 51.49, 46.63, 41.48, 36.29. HRFAB MS, m/z 571.0734 for $[M+H]^+$ (Calcd 571.0730 for $C_{26}H_{24}N_2O_5I$).

3.1.3. Fmoc-Asp(Clt resin)–NH–CH_2–C_6H_4–I (anchoring to Clt resin), 3. To 2.0 g (2.6 mmol) of 2-chlorotrityl chloride (Clt) resin were added Fmoc-Asp–NH–CH_2–C_6H_4–I (0.37 g, 0.65 mmol) in CH_2Cl_2/DMF (2:1, 5.0 ml), and DIEA (0.90 ml, 5.2 mmol). The mixture was agitated for 2 h at 37°C and the solvent was then removed by filtration. MeOH/DIEA (9:1, 8 ml) was added to the resin and the mixture was further agitated for 30 min at 25°C. The resin was filtered, washed with MeOH, and then dried in vacuo to yield 2.2 g of **3**, substituted at a level of 0.22 mmol/g.

3.1.4. Fmoc-Asp(Wang resin)–NH–CH_2–C_6H_4–I (anchoring to Wang resin), 4. To 1.0 g (0.70 mmol) of Wang resin were added Fmoc-Asp–NH–CH_2–C_6H_4–I (1.0 g, 1.8 mmol) in DMF (5.0 ml), DIEA (0.12 ml, 0.70 mmol), DMAP (86 mg, 0.70 mmol), and DIPCDI (0.28 ml, 1.8 mmol), and the mixture was agitated for 2 h at 37°C. The resin was filtered, washed with DMF and MeOH, and then dried in vacuo to yield 1.1 g of **4**, substituted at a level of 0.21 mmol/g.

3.1.5. CH_2=CHCO–Gly-Arg(Mtr)-Gly-Asp(Clt resin)–NH–CH_2–C_6H_4–I (condensation on Clt resin), 5. Piperidine (20%) in DMF was added to the starting resin **3** and the mixture was agitated for 20 min at 25°C. The N^{α}-deprotected resin was filtered and washed with DMF. To this resin, Fmoc-Gly-OH (2.5 equiv.) in DMF, HOBt (2.5 equiv.), DIEA (2.5 equiv.), and DIPCDI (2.5 equiv.) were added and the resin mixture was agitated for 90 min at 25°C. After washing the resin with DMF, the same deprotection and condensation procedure was repeated for the incorporation of Fmoc-Arg(Mtr)-OH, Fmoc-Gly-OH, and acrylic acid monomer. At each condensation step, the results of a Kaiser ninhydrin test were negative after a single coupling reaction. The resin obtained was filtered, washed with DMF and MeOH, and dried to yield 2.3 g (98%) of desired tetrapeptide–Clt resin **5**. Amino acid analysis after 12 M HCl-propionic acid hydrolysis at 110°C for 18 h: Asp 0.97, Gly 1.98, Arg 1.00 (recovery of Arg 80%).

3.1.6. CH_2=CHCO–Gly-Arg(Mtr)-Gly-Asp(Wang resin)–NH–CH_2–C_6H_4–I (condensation on Wang resin). Starting from 2.1 g of the anchored resin **4**, the same procedure as described for Clt resin was used for construction of the peptide chain to yield 2.1 g of product resin. The recovery of Arg after 12M HCl–propionic acid hydrolysis (110°C, 18 h) of the product resin was 5.0%.

3.1.7. Diketodiazepin, 11 and *N*-(fluoren-9-ylmethyl)-piperidine, 12. To 0.50 g of Fmoc-Gly-Asp(Wang resin)–NH–CH_2–C_6H_4–I, **10** (0.27 mmol/g) was added 20% piperidine in DMF (6.0 ml), and the mixture agitated for 40 min at 25°C. The resin was filtered, and washed with DMF. The cleavage yield (63%) was estimated by amino acid analysis after 12 M HCl–propionic acid hydrolysis (66 h at 110°C) of the resin: the ratios of amino acids in the hydrolysate of the resin; Asp 1.00, Gly 0.98 (recovery 33%), and of the starting dipeptide resin; Asp 1.00, Gly 0.95 (recovery 88%).

The solvent of the filtrate was removed by evaporation and the residue was suspended in $CHCl_3$ (3.0 ml). The precipitate of the suspension was washed with $CHCl_3$, Et_2O, and then dried in vacuo to yield 21 mg (62% recovery) of **11** as a solid: Mp 207°C (decomp.) $[\alpha]_D^{25}=8.6°$ (c 0.30, DMSO), ^{1}H NMR (400 MHz, DMSO-d6) (8.45 (br t, J=5.6 Hz, 1H), 7.99 (s, 1H), 7.95 (s, 1H), 7.63 (s, 1H), 7.60 (d, J=7.7 Hz, 1H), 7.28 (d, J=7.7 Hz, 1H), 7.11 (t, J=7.7 Hz, 1H), 4.23 (br d, J=5.5 Hz, 2H), 4.09 (br t, J=5.0 Hz, 1H), 3.72 (s, 2H), 2.63 (d, J=5.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d6) δ169.18, 167.83, 166.02, 142.06, 135.81, 135.49, 130.47, 126.73, 94.76, 51.16, 44.59, 41.38, 37.60. HRFAB MS, m/z 388.0162 for $[M+H]^+$ (Calcd 388.0158 for $C_{13}H_{15}N_3O_3I$).

The solvent of the supernatant was removed by evaporation and the residue was purified by silica gel column chromatography using $CHCl_3$ to yield 18 mg (recovery 80%) of **12** as a solid: Mp. 107–109°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J=7.5 Hz, 2H), 7.69 (d, J=7.5 Hz, 2H), 7.36 (t, J=7.4 Hz, 2H), 7.284 (t, J=7.4 Hz, 1H), 7.281 (t, J=7.4 Hz, 1H), 4.04 (t, J=8.0 Hz, 1H), 2.57 (d, J=8.0 Hz, 2H), 2.56 (br t, J=5.7 Hz, 4H), 1.68 (tt, J=5.5, 5.7 Hz, 4H), 1.51 (tt, J=5.6 Hz, J=5.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.68, 140.96, 126.94, 126.64, 125.42, 119.58, 63.08, 54.91, 44.82, 26.25, 24.63. HRFAB MS, m/z 264.1757 for $[M+H]^+$ (Calcd 264.1752 for $C_{19}H_{22}N$).

3.1.8. CH_2=CHCO–Gly-Arg(Mtr)-Gly-Asp–NH–CH_2–C_6H_4–I, 6. To 0.10 g of RGD–Clt resin **5** (0.21 mmol/g) was added AcOH/TFE/CH_2Cl_2 (1:1:8, 3.0 ml) and the mixture was agitated for 45 min at 25°C. The resin was filtered, washed with AcOH/TFE/CH_2Cl_2 (1:1:8, 3.0 ml). The solvent of the filtrate was removed by evaporation

and the crude product was isolated by precipitation with H_2O to yield 15 mg (80%) of **6** as a solid: Rt_1 17.6 min [CH_3CN (20–80%/30 min)], HRFAB MS, *m/z* 885.2091 for $[M+H]^+$ (Calcd 885.2102 for $C_{34}H_{46}N_8O_{10}SI$). Amino acid analysis after 6 M HCl (110°C, 18 h) hydrolysis; Asp 1.07, Gly 1.88, Arg 1.00.

3.2. Cyclization in solution

9.0 mg (10 μmol) of linear precursor **6** in DMF/H_2O/CH_2Cl_2 (9:1:1, 0.30 ml) was added in one portion to a solution of $Pd(OAc)_2$ (1.0 mg, 4.0 μmol), PPh_3 (1.0 mg, 4.0 μmol), and Bu_4NCl (1.0 mg, 4.0 μmol) in DMF/H_2O/CH_2Cl_2 (9:1:1, 0.50 ml) and the mixture (13 μmol/ml concentration) was stirred at 37°C. A 50 μl aliquot of the solution, taken from the reaction mixture after 2, 4, and 8 h, was added to H_2O (250 μl) and the solution was subjected to analytical HPLC. The results are shown in Fig. 2b.

3.3. Cyclization on solid support

0.30 g (63 μmmol) of CH_2═CHCO–Gly-Arg(Mtr)-Gly-Asp(Clt resin)–NH–CH_2–C_6H_4–I, **5** (0.21 mmol/g) was added in one portion to the solution of $Pd(OAc)_2$ (5.6 mg, 25 μmol), PPh_3 (6.6 mg, 25 μmol), and Bu_4NCl (5.7 mg, 25 μmol) in DMF/H_2O/Et_3N (9:1:1, 3.0 ml) and the mixture (21 μmol/ml concentration) was agitated for 2 h at 37°C. Two other batches of the resin (0.30 g each) were also treated with the same reagent mixture for 4 and 8 h, respectively, at 37°C. Each resin was filtered, washed with DMF and MeOH, and dried in vacuo. To 15 mg of each cyclized resin **7** was added AcOH/TFE/CH_2Cl_2 (1:1:8, 0.50 ml) and the mixture was agitated for 45 min at 25°C. The resin was filtered and the filtrate was evaporated to dryness. The residue was dissolved in 50% AcOH and analyzed by analytical HPLC (Fig. 2a). The desired cyclized product **8** was eluted at 13.7 min [CH_3CN (20–80%/30 min)]: MALDI-TOF MS, 757.407 for $[M+H]^+$ (Calcd 757.297 for $C_{34}H_{45}N_8O_{10}S$). Amino acid analysis after 6 M HCl (110°C, 22 h) hydrolysis: Asp 1.00, Gly 2.05, Arg 0.88.

3.3.1. Cyclic RGD derivative, 9. AcOH/TFE/CH_2Cl_2 (1:1:8, 15 ml) was added to 0.56 g (0.12 mmol) of the cyclized resin **7** (0.22 mmol/g), and the resin mixture was stirred for 45 min at 25°C. The mixture was filtered and the solvent of the filtrate was removed by evaporation. To the residue was added TFA/thioanisole (7.0–0.70 ml) and the mixture was stirred for 3 h at 25°C. The TFA of the mixture was removed by evaporation at 25°C and the residue was extracted with 6 M guanidine HCl (5.0 ml). The product in the extract was purified by preparative HPLC [YMC SH-343-5AM column (20×250 mm), CH_3CN (10–20%/60 min)] to yield 13 mg (overall 20%) of **9** as a white powder: Rt_2 10.4 min [CH_3CN (10–20%)/30 min], ^1H NMR (600 MHz, D_2O) δ7.72 (d, *J*=8.8 Hz, 1H), 7.54 (d, *J*=7.9 Hz, 1H), 7.50 (d, *J*=15.8 Hz, 1H), 7.44 (t, *J*=7.9 Hz, 1H), 7.41 (d, *J*=8.8 Hz, 1H), 7.36 (s, 1H), 7.35 (d, *J*=7.9 Hz, 1H), 6.71 (d, *J*=15.8 Hz, 1H), 4.78 (dd, *J*=9.1, 5.1 Hz, 1H), 4.63 (d, *J*=16.9 Hz, 1H), 4.54 (dd, *J*=5.8, 8.5 Hz, 1H), 4.39 (d, *J*=16.1 Hz, 1H), 4.37 (d, *J*=16.9 Hz, 1H), 4.09 (d, *J*=17.6 Hz, 1H), 4.01 (d, *J*=17.6 Hz, 1H), 3.80 (d, *J*=16.1 Hz, 1H), 3.19 (dd, *J*=6.9, 4.1 Hz, 2H), 2.98 (dd, *J*=16.8 Hz, *J*=5.1 Hz, 1H), 2.86 (dd, *J*=16.8, 9.1 Hz, 1H), 1.94 (tdd, *J*=7.0, 13.9, 8.5 Hz, 1H), 1.71 (tdd, *J*=8.5, 13.9, 5.8 Hz, 1H), 1.59 (tdd, *J*=6.9, 8.5, 7.0 Hz, 2H). HRFAB MS, *m/z* 545.2469 for $[M+H]^+$ (Calcd 545.2472 for $C_{24}H_{33}N_8O_7$). Amino acid analysis after 6 M HCl hydrolysis (110°C, 22 h): Asp 1.00, Gly 2.03, Arg 0.96.

3.4. Alkyl library

Starting from three batches of Fmoc-Asp(Clt resin)–NH–CH_2–C_6H_4–I (0.40 g each of 0.23 mmol/g resin), Fmoc-Gly–OH, Fmoc-γAbu–OH, and Fmoc-εAhx–OH were introduced in parallel by piperidine treatment, followed by DIPCDI coupling, as described for linear precursor resin **5**. The three batches were combined and Fmoc-Arg(Mtr)–OH was introduced to the resin, as described above. The resulting resin was divided to five portions and Fmoc-Gly–OH, Fmoc-Ala–OH, Fmoc-αAbu–OH, Fmoc-Nva–OH, and Fmoc-Pro–OH were introduced to the five portions of the resin in parallel. These resins were again combined and acrylic acid was introduced to yield 1.3 g of library precursor resin.

20 mg of the library resin was treated with AcOH/TFE/CH_2Cl_2 (1:1:8, 0.50 ml) for 30 min at 25°C. The resin was filtered and the filtrate was evaporated to dryness. The residue was dissolved in DMF/H_2O (2:1, 150 μl) and a 20 μl aliquot of the solution was subjected to analytical HPLC [CH_3CN (30–70%/30 min)] (Fig. 4a). 1 μl of the eluate of each peak was analyzed by MALDI-TOF MS. [Gly^1, γAbu^3] derivative (Rt_3 16.43 min) 913.086 for $[M+H]^+$ (Calcd 913.792 for $C_{36}H_{50}N_8O_{10}SI$); [Gly^1, Gly^3] derivative (Rt_3 16.9 min) 885.336 for $[M+H]^+$ (Calcd 885.740 for $C_{34}H_{46}N_8O_{10}SI$); [Ala^1, γAbu^3] derivative (Rt_3 17.20 min) 927.573 for $[M+H]^+$ (Calcd 927.818 for $C_{37}H_{52}N_8O_{10}SI$); [Gly^1, ϵAhx^3] derivative (Rt_3 17.56 min) 941.54 for $[M+H]^+$ (Calcd 941.844 for $C_{38}H_{54}N_8O_{10}SI$); [Ala^1, Gly^3] derivative (Rt_3 17.76 min) 899.074 for $[M+H]^+$ (Calcd 899.766 for $C_{35}H_{48}N_8O_{10}SI$); [Pro^1, γAbu^3] derivative (Rt_3 17.98 min) 953.32 for $[M+H]^+$ (Calcd 953.854 for $C_{39}H_{54}N_8O_{10}SI$); [αAbu^1, γAbu^3] derivative (Rt_3 18.32 min) 941.457 for $[M+H]^+$ (Calcd 941.844 for $C_{38}H_{54}N_8O_{10}SI$); [Ala^1, ϵAhx^3] derivative (Rt_3 18.32 min) 955.462 for $[M+H]^+$ (Calcd 955.870 for $C_{39}H_{56}N_8O_{10}SI$); [Pro^1, Gly^3] derivative (Rt_3 18.32 min) 925.457 for $[M+H]^+$ (Calcd 925.802 for $C_{37}H_{50}N_8O_{10}SI$); [αAbu^1, Gly^3] derivative (Rt_3 18.94 min) 913.451 for $[M+H]^+$ (Calcd 913.792 for $C_{36}H_{50}N_8O_{10}SI$); [Pro^1, ϵAhx^3] derivative (Rt_3 18.94 min) 981.544 for $[M+H]^+$ (Calcd 981.906 for $C_{41}H_{58}N_8O_{10}SI$); [αAbu^1, ϵAhx^3] derivative (Rt_3 19.38 min) 969.52 for $[M+H]^+$ (Calcd 969.896 for $C_{40}H_{58}N_8O_{10}SI$); [Nva^1, γAbu^3] derivative (Rt_3 20.07 min) 954.968 for $[M+H]^+$ (Calcd 955.870 for $C_{39}H_{56}N_8O_{10}SI$); [Nva^1, Gly^3] derivative (Rt_3 20.68 min) 927.477 for $[M+H]^+$ (Calcd 927.818 for $C_{37}H_{52}N_8O_{10}SI$); [Nva^1, ϵAhx^3] derivative (Rt_3 21.07 min) 983.511 for $[M+H]^+$ (Calcd 983.922 for $C_{41}H_{60}N_8O_{10}SI$).

0.40 g (76 μmol) of the above library precursor resin was added in one portion to a solution of $Pd(OAc)_2$ (17 mg, 76 μmol), Ph_3P (20 mg, 76 μmol), and Bu_4NCl (17 mg, 76 μmol) in DMF/H_2O/Et_3N (9:1:1, 1.5 ml) and the mixture (50 μmol/ml concentration) was agitated for 5 h at 37°C.

The resin was filtered, washed with DMF and MeOH, and dried in vacuo. 20 mg of the cyclized resin was treated with AcOH/TFE/CH_2Cl_2 and analyzed by HPLC- MALDI-TOF MS, as described for library precursor resin (Fig. 4b). [Gly^1, γAbu^3] derivative (Rt_3 8.17 min) 785.23 for $[M+H]^+$ (Calcd 785.88 for $C_{36}H_{49}N_8O_{10}S$); [Gly^1, Gly^3] derivative (Rt_3 8.91 min) 757.36 for $[M+H]^+$ (Calcd 757.828 for $C_{34}H_{45}N_8O_{10}S$); [Gly^1, ϵAhx^3] derivative (Rt_3 9.62 min) 813.652 for $[M+H]^+$ (Calcd 813.932 for $C_{38}H_{53}N_8O_{10}S$); [Ala^1, γAbu^3] derivative (Rt_3 9.62 min) 799.593 for $[M+H]^+$ (Calcd 799.906 for $C_{37}H_{51}N_8O_{10}S$); [Ala^1, Gly^3] derivative (Rt_3 10.00 min) 771.443 for $[M+H]^+$ (Calcd 771.854 for $C_{35}H_{47}N_8O_{10}S$); [Ala^1, ϵAhx^3] derivative (Rt_3 10.07 min) 827.569 for $[M+H]^+$ (Calcd 827.958 for $C_{39}H_{55}N_8O_{10}S$); [Pro^1, Gly^3] derivative (Rt_3 10.07 min) 797.519 for $[M+H]^+$ (Calcd 797.890 for $C_{37}H_{49}N_8O_{10}S$); [αAbu^1, γAbu^3] derivative (Rt_3 11.91 min) 813.543 for $[M+H]^+$ (Calcd 813.932 for $C_{38}H_{53}N_8O_{10}S$); [αAbu^1, Gly^3] derivative (Rt_3 12.48 min) 785.136 for $[M+H]^+$ (Calcd 785.880 for $C_{36}H_{49}N_8O_{10}S$); [Pro^1, γAbu^3] derivative (Rt_3 12.48 min) 825.186 for $[M+H]^+$ (Calcd 825.942 for $C_{39}H_{53}N_8O_{10}S$); [αAbu^1, ϵAhx^3] derivative (Rt_3 12.48 min) 841.222 for $[M+H]^+$ (Calcd 841.984 for $C_{40}H_{57}N_8O_{10}S$); [Pro^1, ϵAhx^3] derivative (Rt_3 12.48 min) 853.255 for $[M+H]^+$ (Calcd 853.994 for $C_{41}H_{57}N_8O_{10}S$); [Nva^1, γAbu^3] derivative (Rt_3 14.72 min) 827.58 for $[M+H]^+$ (Calcd 827.958 for $C_{39}H_{55}N_8O_{10}S$); [Nva^1, ϵAhx^3] derivative (Rt_3 15.07 min) 855.866 for $[M+H]^+$ (Calcd 856.01 for $C_{41}H_{59}N_8O_{10}S$); [Nva^1, Gly^3] derivative (Rt_3 15.24 min) 799.526 for $[M+H]^+$ (Calcd 799.906 for $C_{37}H_{51}N_8O_{10}S$).

To 80 mg (15 μmol) of the above cyclized library resin was added AcOH/TFE/CH_2Cl_2 (1:1:8, 2.0 ml), and the mixture was stirred for 40 min at 25°C. The mixture was filtered and the filtrate was evaporated to dryness. To the residue was added TFA/thioanisole (2.0–0.20 ml), and the mixture was stirred for 3 h at 25°C. The TFA of the mixture was removed by evaporation at 25°C and the residue was extracted with 4 M AcOH (2.0 ml). The aqueous phase was washed with Et_2O (2.0 ml), filtered, and then lyophilized. The resulting powdered product was partially purified by semipreparative HPLC [CH_3CN (10–30%/60 min)]: the product which eluted below 25% concentration of CH_3CN was pooled and lyophilized to yield 0.90 mg (10%) of a white powder. Each component was identified by HPLC[CH_3CN (10–30%/30 min)]- MALDI-TOF MS analysis (Fig. 5). [Gly^1, γAbu^3] derivative (Rt_4 15.22 min) 573.353 for $[M+H]^+$ (Calcd 573.620 for $C_{26}H_{37}N_8O_7$); [Ala^1, γAbu^3] derivative (Rt_4 16.59 min) 587.452 for $[M+H]^+$ (Calcd 587.646 for $C_{27}H_{39}N_8O_7$); [Gly^1, Gly^3] derivative (Rt_4 16.92 min) 545.418 for $[M+H]^+$ (Calcd 545.568 for $C_{24}H_{33}N_8O_7$); [Gly^1, ϵAhx^3] derivative (Rt_4 18.47 min) 601.503 for $[M+H]^+$ (Calcd 601.672 for $C_{28}H_{41}N_8O_7$); [Ala^1, Gly^3] derivative (Rt_4 18.47 min) 559.426 for $[M+H]^+$ (Calcd 559.594 for $C_{25}H_{35}N_8O_7$); [Pro^1, Gly^3] derivative (Rt_4 19.68 min) 584.957 for $[M+H]^+$ (Calcd 585.630 for $C_{27}H_{37}N_8O_7$); [Ala^1, ϵAhx^3] derivative (Rt_4 20.08 min) 615.228 for $[M+H]^+$ (Calcd 615.698 for $C_{29}H_{43}N_8O_7$); [αAbu^1, γAbu^3] derivative (Rt_4 20.08 min) 601.433 for $[M+H]^+$ (Calcd 601.672 for $C_{28}H_{41}N_8O_7$); [Pro^1, γAbu^3] derivative (Rt_4 21.43 min) 613.386 for $[M+H]^+$ (Calcd 613.682 for $C_{29}H_{41}N_8O_7$); [αAbu^1, Gly^3] derivative (Rt_4 22.18 min) 573.28 for $[M+H]^+$ (Calcd 573.620 for $C_{26}H_{37}N_8O_7$); [Pro^1, ϵAhx^3] derivative (Rt_4 22.87 min) 641.451 for $[M+H]^+$ (Calcd 641.734 for $C_{31}H_{45}N_8O_7$); [αAbu^1. ϵAhx^3] derivative (Rt_4 22.87 min) 627.849 for $[M+H]^+$ (Calcd 628.716 for $C_{30}H_{44}N_8O_7$); [Nva^1, γAbu^3] derivative (Rt_4 24.64 min) 615.19 for $[M+H]^+$ (Calcd 615.698 for $C_{29}H_{43}N_8O_7$); [Nva^1, Gly^3] derivative (Rt_4 26.78 min) 587.225 for $[M+H]^+$ (Calcd 587.646 for $C_{27}H_{39}N_8O_7$); [Nva^1, ϵAhx^3] derivative (Rt_4 26.78 min) 643.247 for $[M+H]^+$ (Calcd 643.750 for $C_{31}N_{47}N_8O_7$).

3.5. Functional group library

Three batches of Fmoc-Asp(Clt resin)–NH–CH_2–C_6H_4–I (0.40 g each, 0.23 mmol/g resin) were used as the starting resins. Fmoc-Gly-OH, Fmoc-γAbu-OH, and Fmoc-εAhx-OH at position 4, and Fmoc-Ser(Bu^t)-OH, Fmoc-Dpr(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(OBu^t)-OH, and Fmoc-Asn(Trt)-OH at position 1 were incorporated by the split and mix method as described for the alkyl library to yield 1.3 g of the precursor library resin. An aliquot of the library resin was cleaved with AcOH/TFE/CH_2Cl_2 (1:1:8) and each component was identified by HPLC[CH_3CN (30–70%/30 min)]-HRFAB MS analysis. [Ser(Bu^t)^1, γAbu^3] derivative (Rt_3 21.91 min) 999.3152 for $[M+H]^+$ (Calcd 999.3147 for $C_{41}H_{60}O_{11}N_8SI$); [Dpr(Boc)^1, γAbu^3] derivative (Rt_3 22.27 min) 1042.3196 for $[M+H]^+$ (Calcd 1042.3205 for $C_{42}H_{61}O_{12}N_9SI$); [Ser(Bu^t)^1, Gly^3] derivative (Rt_3 22.50 min) 971.2841 for $[M+H]^+$ (Calcd 971.2834 for $C_{39}H_{56}O_{11}N_8SI$); [Ser(Bu^t)^1, ϵAhx^3] derivative (Rt_3 22.83 min) 1027.3472 for $[M+H]^+$ (Calcd 1027.3460 for $C_{43}H_{64}O_{11}N_8SI$); [Dpr(Boc)^1, Gly^3] derivative (Rt_3 22.83 min) 1014.2876 for $[M+H]^+$ (Calcd 1014.2892 for $C_{40}H_{57}O_{12}N_9SI$); [Asp(OBu^t)^1, γAbu^3] derivative (Rt_3 22.93 min) 1027.3088 for $[M+H]^+$ (Calcd 1027.3096 for $C_{42}H_{60}O_{12}N_8SI$); [Dpr(Boc)^1, ϵAhx^3] derivative (Rt_3 23.16 min) 1070.3514 for $[M+H]^+$ (Calcd 1070.3518 for $C_{44}H_{65}O_{12}N_9SI$); [Lys(Boc)^1, γAbu^3] drivative (Rt_3 23.27 min) 1084.3665 for $[M+H]^+$ (Calcd 1084.3675 for $C_{45}H_{67}O_{12}N_9SI$); [Asp(OBu^t)^1, Gly^3] derivative (Rt_3 23.50 min) 999.2765 for $[M+H]^+$ (Calcd 999.2783 for $C_{40}H_{56}O_{12}N_8SI$); [Lys(Boc)^1, Gly^3] derivative (Rt_3 23.92 min) 1056.3373 for $[M+H]^+$ (Calcd 1056.3362 for $C_{43}H_{63}O_{12}N_9SI$); [Asp(OBu^t)^1, ϵAhx^3] derivative (Rt_3 23.92 min) 1055.3414 for $[M+H]^+$ (Calcd 1055.3409 for $C_{44}H_{64}O_{12}N_8SI$); [Lys(Boc)^1, ϵAhx^3] derivative (Rt_3 24.14 min) 1112.4023 for $[M+H]^+$ (Calcd 1112.3988 for $C_{47}H_{71}O_{12}N_9SI$); [Asn(Trt)^1, γAbu^3] derivative (Rt_3 31.39 min) 1212.3714 for $[M+H]^+$ (Calcd 1212.3726 for $C_{57}H_{67}O_{11}N_9SI$); [Asn(Trt)^1, Gly^3] derivative (Rt_3 31.39 min) 1184. 3420 for $[M+H]^+$ (Calcd 1184.3413 for $C_{55}H_{63}O_{11}N_9SI$); [Asn(Trt)^1, ϵAhx^3] derivative (Rt_3 31.82 min) 1240.4065 for $[M+H]^+$ (Calcd 1240.4039 for $C_{59}H_{71}O_{11}N_9SI$).

Cyclization of the precursor resin (0.40 g, 76 μmol) and the HPLC[CH_3CN (30–70%/30 min)]-HRFAB MS analysis were carried out using the same procedure as described for the alkyl library. [Ser(Bu^t)^1, γAbu^3] derivative (Rt_3 17.24 min) 871.4006 for $[M+H]^+$ (Calcd 871.4024 for $C_{41}H_{59}O_{11}N_8S$); [Ser(Bu^t)^1, ϵAhx^3] derivative (Rt_3 17.60 min) 899.4359 for $[M+H]^+$ (Calcd 899.4337 for

$C_{43}H_{63}O_{11}N_8S$); [Ser(Bu^t)^1, Gly^3] derivative (Rt_3 17.92 min) 843.3719 for $[M+H]^+$ (Calcd 843.3711 for $C_{39}H_{55}O_{11}N_8S$); [Dpr(Boc)^1, Gly^3] derivative (Rt_3 17.92 min) 886.3779 for $[M+H]^+$ (Calcd 886.3769 for $C_{40}H_{56}O_{12}N_9S$); [Dpr(Boc)^1, γAbu^3] derivative (Rt_3 17.92 min) 914.4070 for $[M+H]^+$ (Calcd 914.4082 for $C_{42}H_{60}O_{12}N_9S$); [Dpr(Boc)^1, ϵAhx^3] derivative (Rt_3 17.92 min) 942.4366 for $[M+H]^+$ (Calcd 942.4395 for $C_{44}H_{64}O_{12}N_9S$); [Asp(OBu^t)^1, Gly^3] derivative (Rt_3 18.38 min) 871.3668 for $[M+H]^+$ (Calcd 871.3660 for $C_{40}H_{55}O_{12}N_8S$); [Asp(OBu^t)^1, γAbu^3] derivative (Rt_3 18.38 min) 899.3984 for $[M+H]^+$ (Calcd 899.3973 for $C_{42}H_{59}O_{12}N_8S$); [Asp(OBu^t)^1, ϵAhx^3] derivative (Rt_3 18.58 min) 927.4303 for $[M+H]^+$ (Calcd 927.4286 for $C_{44}H_{63}O_{12}N_8S$); [Lys(Boc)^1, γAbu^3] derivative (Rt_3 18.94 min) 956.4570 for $[M+H]^+$ (Calcd 956.4552 for $C_{45}H_{66}O_{12}N_9S$); [Asn(Trt)^1, ϵAhx^3] derivative (Rt_3 18.94 min) 1112.4366 for $[M+H]^+$ (Calcd 1112.4916 for $C_{59}H_{70}O_{11}N_9S$); [Lys(Boc)^1, Gly^3] derivative (Rt_3 19.22 min) 928.4230 for $[M+H]^+$ (Calcd 928.4239 for $C_{43}H_{62}O_{12}N_9S$); [Lys(Boc)^1, ϵAhx^3] derivative (Rt_3 19.22 min) 984.4874 for $[M+H]^+$ (Calcd 984.4865 for $C_{47}H_{70}O_{12}N_9S$); [Asn(Trt)^1, Gly^3] derivative (Rt_3 19.22 min) 1056.5254 for $[M+H]^+$ (Calcd 1056.4290 for $C_{55}H_{62}O_{11}N_9S$); [Asn(Trt)^1, γAbu^3] derivative (Rt_3 19.22 min) 1084.3840 for $[M+H]^+$ (Calcd 1084.4603 for $C_{57}H_{66}O_{11}N_9S$).

Cleavage and deprotection of the cyclized resin (50 mg, 10 μmol) were carried out using the same procedure as described for the alkyl library. The crude product was partially purified by semipreparative HPLC [CH_3CN (10–30%/60 min)]: the product, which eluted below 20% concentration of CH_3CN was pooled and lyophilized to yield 0.50 mg (9.0%) of a white powder. Each component was identified by HPLC [CH_3CN (10–30%/60 min)]-HRFAB MS analysis (Fig. 7). [Dpr^1, γAbu^3] derivative (Rt_5 11.32 min) 602.3055 for $[M+H]^+$ (Calcd 602.3051 for $C_{27}H_{40}O_7N_9$); [Dpr^1, Gly^3] derivative (Rt_5 11.32 min) 574.2744 for $[M+H]^+$ (Calcd 574.2738 for $C_{25}H_{36}O_7N_9$); [Lys^1, γAbu^3] derivative (Rt_5 14.51 min) 644.3526 for $[M+H]^+$ (Calcd 644.3520 for $C_{30}H_{46}O_7N_9$); [Lys^1, Gly^3] derivative (Rt_5 15.70 min) 616.3212 for $[M+H]^+$ (Calcd 616.3207 for $C_{28}H_{42}O_7N_9$); [Dpr^1, ϵAhx^3] derivative (Rt_5 16.51 min) 630.3358 for $[M+H]^+$ (Calcd 630.3364 for $C_{29}H_{44}O_7N_9$); [Asn^1, γAbu^3] derivative (Rt_5 17.15 min) 630.3005 for $[M+H]^+$ (Calcd 630.3000 for $C_{28}H_{40}O_8N_9$); [Ser^1, γAbu^3] derivative (Rt_5 18.12 min) 603.2885 for $[M+H]^+$ (Calcd 603.2891 for $C_{27}H_{39}O_8N_8$); [Asn^1, Gly^3] derivative (Rt_5 18.83 min) 602.2675 for $[M+H]^+$ (Calcd 602.2687 for $C_{26}H_{36}O_8N_9$); [Ser^1, Gly^3] derivative (Rt_5 19.42 min) 575.2588 for $[M+H]^+$ (Calcd 575.2578 for $C_{25}H_{35}O_8N_8$); [Asp^1, γAbu^3] derivative (Rt_5 20.18 min) 631.2833 for $[M+H]^+$ (Calcd 631.2840 for $C_{28}H_{39}O_9N_8$); [Lys^1, ϵAhx^3] derivative (Rt_5 20.18 min) 672.3843 for $[M+H]^+$ (Calcd 672.3833 for $C_{32}H_{50}O_7N_9$); [Asp^1, Gly^3] derivative (Rt_5 22.51 min) 603.2517 for $[M+H]^+$ (Calcd 603.2527 for $C_{26}H_{35}O_9N_8$); [Asn^1, ϵAhx^3] derivative (Rt_5 23.35 min) 658.3320 for $[M+H]^+$ (Calcd 658.3313 for $C_{30}H_{44}O_8N_9$); [Ser^1, ϵAhx^3] derivative (Rt_5 24.34 min) 631.3212 for $[M+H]^+$ (Calcd 631.3204 for $C_{29}H_{43}O_8N_8$); [Asp^1, ϵAhx^3] derivative (Rt_5 26.44 min) 659.3163 for $[M+H]^+$ (Calcd 659.3153 for $C_{30}H_{43}O_9N_8$).

3.6. Inhibition assay of fibrinogen binding to immobilized GPIIb/IIIa

Human GpIIb/IIIa (Enzyme Research Lab.) was diluted with Tris buffer containing 20 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$ and 0.02% NaN_3 (final concentration was 5 mg/ml). The solution was then immediately added to 96-well microtiter plates at 100 μl per well. Each well was washed with 50 mM Tris, 100 mM NaCl, 2 mM $CaCl_2$, and 0.02% NaN_3 (pH 7.4) and then incubated with EIA buffer containing 35 mg/ml bovine serum albumin for 3 h at room temperature. After each well was washed with EIA buffer containing 1 mg/ml BSA, fibrinogen solution (30 μg/ml) was added to each well and incubated for 3 h at room temperature in the presence or absence of peptides. Following the incubation each well was washed twice with 400 μl of EIA buffer. Bound fibrinogen was quantitated by the addition of 100 μl of an anti-human fibrinogen antibody conjugated to alkaline phosphatase (1:1000 dilution, EY laboratories), followed by a wash with EIA buffer and the addition of *p*-nitrophenyl phosphate (*p*NPP Tablets, Sigma). The resulting IC_{50} value was 2.0×10^{-5} M.

3.7. Molecular dynamics simulations

The ROESY experiments were carried out using mixing time 300 ms with a spin locking field 30 dB. The spectral width was 4085 Hz. Molecular dynamics (MD) simulation were done using the InsightII/Discover program (Biosym Technologies) on an INDY3 computer. The simulations were done using the consistent valence force field (CVFF). MD simulation for 50 ps at 1000 K was carried out. Every 10 fs (20 steps) a structure was stored in the history file for analysis. The history was analyzed by plotting the energy against time. Twelve conformers those provided local minimum in the plot were sampled as a starting conformer of next MD simulation. These conformers were subjected to the 300 K MD simulation with 17 ROE restrains for 10 ps. Every 1 ps (1000 steps) a structure was stored in the history file. Then the 132 conformers were subjected to unrestrained minimization using the VA09A algorithm until the maximum derivative was less than 0.001 kcal/mol. An error of ±30° was tolerated for the phi angles calculated from J(NH–Ha) of 2Arg and 4Asp. The conformers that were inconsistent with this threshold were discarded. Ninety-seven conformers were analyzed. These conformers were clustered into three groups in their 2Arg–4Asp phi torsion angle plot. Each group has another total energies (81.3, 82.1,85.6 averaged, relatively) with small standard deviation (SD=1.33, 1.87, 1.28). In low-energy group, conformers have phi angle around −120° and −80° in 2Arg and 4Asp. Distance between guanidino carbon of 2Arg and carbonyl carbon of 4Asp was 11.77 A (averaged) with SD=1.54. Details of the procedure and results will be published elsewhere.

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